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13. ABSTRACT (Maximum 200 Words)

A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer. The genetic factors responsible are largely unknown, although the autosomal dominant, relatively high penetrant genes BRCA1/2 may account for 3%. It has been hypothesized that susceptibility genes of lower penetrance may also affect breast cancer risk, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen. Previous studies of these susceptibility genes have not been conducted with women with high familial risk. This study is being conducted with identical twins with differing genetic risks (i.e. concordant for breast cancer pairs vs. discordant pairs) as well as unaffected controls. We have chosen to focus on those genes related to estrogen metabolism and carcinogen metabolism. In the estrogen metabolism pathway, polymorphisms have been described related to the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 gene. Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. We will compare the frequency of selected polymorphisms in these genes in 200 breast cancer concordant, 200 discordant, and 200 control women. We currently have tissue or buccal smears and informed consents from 130 concordant, 152 discordant, and 133 control women. Laboratory analyses of the CYP17 gene have shown some inconsistencies with repeat testing and additional testing is being done to assure that the results are accurate. Once the assay method has been validated, additional genes will be tested..

14. SUBJECT TERMS

CYP17, CYP19, COMT, CYP1A1, HSD17B1, GSTM1, GSTP1, Twins, estrogen metabolism, carcinogen metabolism, genetics

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4) INTRODUCTION

A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer(1), and this is true for postmenopausal as well as the premenopausal cases, among which the autosomal dominant, relatively high penetrant genes BRCA1 and BRCA2 are most prominent(2). It has been hypothesized that susceptibility genes of lower penetrance are more prevalent than among the latter, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen (3), the common factor underlying most known predictors of breast cancer risk (4) (5) (6). Recent reviews have identified several candidate genes (7) (8) (9). We have chosen to focus on those genes related to estrogen metabolism and carcinogen metabolism.

In the estrogen metabolism pathway, four genetic polymorphisms have been described related to the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 (or also called the EDH17B2) gene. For example, a polymorphism (called A2) on the CYP17 gene has recently been linked to higher endogenous estrogen levels and an earlier age at menarche (10). The same polymorphism was linked to increased risk of aggressive breast cancer, although one attempt to confirm this finding was unsuccessful(11). Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. These studies, however, have not been conducted with women known to be at high familial risk, where the prevalence of the polymorphism may be expected to be higher, if it is associated with the development of breast cancer. This study proposes to take advantage of a unique subset of very high risk women in whom cumulative exposure to endogenous estrogen may play an especially important role in breast cancer etiology.

The identification of families to study these inherited genetic factors is more difficult because of the anticipated lower penetrance of the candidate genes and occurrence of more sporadic cases, especially among older women. The International Twin Study includes both breast cancer concordant and discordant identical twin pairs. The concordant MZ twin pairs represent families with a very high familial risk of breast cancer, while the MZ discordant twins are likely to represent non-heritable cancer. We plan to obtain DNA from subsets of these pairs as well as from control women without breast cancer (and without a family history of breast cancer) and to test for the genetic polymorphisms specified to determine if any are differentially associated with cases from twins with a high likelihood of heritable breast cancer (i.e. those from identical concordant pairs). This study should provide important clues regarding other genetic factors that may be associated with breast cancer etiology. Initial work on the project and the CYP17 laboratory work was funded under a grant from the California Breast Cancer Research Project (CA-BCRP).

A recent publication by the P.I. on epidemiological risk factors within the concordant for breast cancer identical twins, who are presumed to have a high genetic susceptibility, has indicated that factors associated with the onset of hormones at puberty may be especially critical (33) (also included in Appendix). The DNA from these twin pairs will be especially valuable in identifying additional genetic factors (and combinations of them) that may be related to breast cancer.

Other studies have relied on family history of breast cancer to identify women at high genetic risk, however this method may not be able to select for combinations of genetic factors in which two or more genes interact to increase risk. In these circumstances, the genes may be derived from both sides of the family, neither with a family history for breast cancer. Identical twins with both having breast cancer represent a group with high genetic susceptibility regardless of family history. Furthermore, since they are identical genetically, they also offer the opportunity to study gene x environment interaction. From the recently published study (33), it appeared that the earlier the puberty occurred the higher the risk of first breast cancer in the pair.

5) BODY

Technical Objectives and Work Accomplished in year 3:

Task 1: To complete follow-up of female identical twin pairs with breast cancer (Months 1-18)

1. *Continue follow-up begun under CA-BCRP grant*
2. *Hire Programmer, set up tracking database*
3. *Continue to mail follow-up forms with return envelope to last known address of twins. Enter data from responses.*
4. *Submit nonrespondent names to National Death Index.*
5. *Submit names of nonrespondent twins not known to be deceased to TRW/ Experian to obtain updated addresses. Resend follow-up forms.*
6. *Continue follow-up by phone calls, internet searches, and contact with relatives.*

It was previously reported that a data file was created from the International Twin Registry that selected all of the identical female twin pairs in which one or both members had been diagnosed with breast cancer. In total there are 1,491 identical pairs in this database and 1,199 of them were initially classified as discordant pairs, 263 as concordant, and 29 of uncertain concordance. A follow-up form was sent to all living members of all of the discordant pairs, and new breast cancers have been reported in the previously healthy twin of 62 of these pairs. Thus as a result of this information, there are now 338 concordant pairs and 1,153 discordant pairs. Follow-up efforts have consisted of mailing 1,883 follow-up forms to living twins in these pairs, and 1,029 have been returned completed. 260 were returned by the post office and 478 were not returned by either the twin or the post office. Tracing efforts were implemented to locate the nonrespondents. Follow-up of all nonrespondents will continue using the National Death Index. (This component was funded under the CA-BCRP grant).

Task 2: Identify new breast cancers and obtain medical record documentation and tissue blocks. (Months 6-20)

1. *When new breast cancer is identified, obtain medical consent form from twin or next of kin, and request records and tissue blocks from hospital*
2. *Follow-up requests with hospitals*

The goal of the study is to obtain genomic DNA from at least one member of 200 of the concordant pairs, from the case in 200 of the discordant pairs, and from 200 control women without a personal or family history of breast cancer. From a previous study, tissue blocks have

been obtained from some of the breast cancer pairs (concordant and discordant). As a result of the follow-up effort, we have identified 62 previously discordant pairs in whom the unaffected member has developed breast cancer. Thus the number of concordant and discordant pairs has been adjusted to reflect the current status.

To participate in the study, the eligible participants are sent a letter describing the study along with the informed consent documents. Our study manager then calls the twin to go over the informed consent with her over the telephone. Then if she agrees to participate and donate the required tissue to the study, she then signs the informed consent form and mails it back to us. .

As of this time (7/23/03) the current numbers of MZ twins (and controls) in each subset with tissue and signed consent forms is the following:

	Concordant	Discordant	Controls
Number identified and either has agreed to participate or is still a potential participant*	179	979	133**
DOD consent signed and tissue/buccal smear available	**130**	**152**	**133**
(Number of above with buccal smear)	(26)	(12)	(133)
Goal	200	200	200
Additional cases/controls needed to reach Goal	**70**	**48**	**67**
Tissue available (and still attempting obtain signed informed consent)*	19	37	
Additional cases who could be sent buccal smear kit	30	788	
Potential subjects	49	825	

*after elimination of refusals, and deceased cases with no available tissue. Reasons for refusal included not interested, and too busy as well as the language that the DOD requires us to include in the informed consent regarding 'POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH'. The P.I. however is planning to recontact some of the 'soft' refusals, send them a copy of the recently published New England Journal article (33) and emphasize the importance of the study.

**this number increases with the addition of new cases

We currently have tissue or buccal smears and signed DOD informed consents for 130 concordnat pairs, 152 discordant pairs and 133 controls. We will have no trouble reaching our target of 200 discordant pairs and 200 controls, however, at the moment our total potential number or concordant pairs is 179. We are still hoping to convert some of the soft refusals in this group.

Task 3: Obtain buccal smears from living member of case pairs when blocks not available (Months 1-20)

1. *If tissue blocks are no longer available from either member of the case pairs and there is a living twin, send letter to obtain buccal smear.*
2. *Send buccal smear kit and return mailing supplies and postage to these individuals.*

The procedures for obtaining buccal smears have been developed and kits have been assembled for this purpose. We are using Epicentre Technologies Master Amp Buccal Swab Brush. Two brushes are being sent to the selected cases (and controls) and they are asked to use one for each cheek. Once the swabs are returned to us they are being kept frozen until the laboratory analyses are done. To date we have collected buccal smears from 26 concordant pairs, 12 discordant pairs and 133 controls.

Task 3: Identify 200 control women and obtain buccal smear and risk factor questionnaire from each of them
(Months 1-20)

1. *Contact case pairs to obtain listing of unrelated breast cancer free potential control women selected from sisters-in-laws and friends.*
2. *Randomly select a women from this list and mail introductory letter.*
3. *Obtain buccal smear and risk factor questionnaire from each control woman through the mail.*

We have developed the protocol for selecting controls and this is working well. To date we have identified 133 controls and have obtained the buccal smear and short risk factor questionnaire from all of them.

Task 4: Laboratory analysis of DNA from tissue and buccal smears to identify polymorphisms in the specified breast susceptibility candidate genes
(Months 1-24)

1. *Finish CYP-17 analysis at Dr. Dubeau's Laboratory.*
2. *Extract additional DNA as necessary for the additional genetic tests.*
3. *Do additional tests for CYP19, COMT, HSD17B1, GSTM1, GSTP1, and CYP1A1.*
4. *Receive results and enter data into database.*
5. *Store tissue for future genetic studies.*

We have had some difficulties in this area and are working to resolve the problems. This has caused some delay in reporting of the results and we have requested a 1 year no cost extension as a result. We noticed some inconsistencies in some repeat samples. This may have been due to low concentration of DNA obtained from the archived tissue blocks. Dr. Dubeau developed a linear PCR method that involved a linear amplification during the first PCR step. He repeated the assay on some samples multiple times. We have also consulted with Rob Fannon of BioServe Biotechnologies, Ltd. 1050 West Street, Laurel, Maryland 20707. This company used the MALDI-TOF method which may be superior for samples with a low yield of DNA. Dr. Dubeau is planning to do DNA sequencing for samples that have provided inconsistent results. Once the reasons for the inconsistencies have been resolved, using the CYP17 gene as the test assay, we will proceed rapidly to produce the results for the other genes that we plan to study, since the DNA is being extracted and can be used for multiple tests.

Task 4 Data analysis (Months 18-32)

1. *Link data on genetic factors to other information from twins and controls including risk factor information and other tumor related information when available (e.g. ER positivity)*
2. *Complete analyses of data to determine relationship of the specified polymorphisms to breast cancer susceptibility.*
3. *Submit papers and reports.*

Based on the inconsistencies in CYP17 results described above, we are not ready to proceed with the data analysis at this time. However, we anticipate that these problems will be resolved shortly and the results will be available.

6) Key Research Accomplishments

a. We have obtained DNA and signed consent forms for 130 concordant pairs, 152 discordant pairs, and 133 controls.
b. DNA has been extracted from buccal smear samples.
c. We are testing the CYP17 assay multiple times, using different techniques to assure that the results are accurate..

7) Reportable Outcomes—none at this time.

8) Conclusions

We anticipate meeting our goals of obtaining DNA from 200 discordant pairs and 200 controls, but may only obtain 175 samples from concordant pairs. Nevertheless this should be sufficient to reach our research goals. We have had some unexpected problems with the PCR assays for CYP17, finding some inconsistencies in repeated samples. Thus we have spent additional time investigating the reasons for the discrepancies with both Dr. Dubeau and BioServe Biotechnologies, LTD. in order to develop a method that produces reliable results. Part of the problem may lie in the quality of the DNA that we have to work with for some of the samples. As a result, we will develop a method for identifying the samples that may not be useable. We are also planning to obtain buccal smears from living twins for whom we already have tissue blocks. The comparison of the results from the buccal smear and the tissue block should help to resolve this issue as well. We anticipate that these problems should be resolved within the next

few months and the analysis of CYP17 and the other assays will proceed quickly. Due to the clues from our recent publication regarding the importance of the puberty for the development of heritable breast cancer (33), we are also investigating other genes which may have relevance to this critical time period.

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10) APPENDICES

Current IRB approval

Copy of Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins.New England Journal of Medicine. 2003; 348:2313-22.

Proposal #001034
Review Category: C

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Date: 6/2/2003
To: **Ann S Hamilton, Ph.D.**
Assistant Professor
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TITLE OF PROPOSAL:
BREAST CANCER SUSCEPTIBILITY GENES IN HIGH RISK WOMEN (DODBCRP)

Action Date: **5/20/2003** Action Taken: **Approved**
Committee: Institutional Review Board No. 3
Note:

The Continuing Review form, response to IRB stipulations from 4/15/03, Lifestyle and Reproductive History Questionnaire (dated 8/21/01) and the revised informed consent documents (dated 4/30/03) were reviewed and discussed by Institutional Review Board #3 (registration #00002881) at the convened meeting on May 20, 2003. This material was distributed to all members for their review prior to the meeting. The primary reviewer received a copy of the IRB file. The complete file was available for reference. Continuation of the study for 1 year was APPROVED by IRB #3 (6 votes for; 0 against; 0 abstentions). ***APPROVAL FOR THIS STUDY IS VALID MAY 20, 2003 TO APRIL 15, 2004.***

The attached IRB approved informed consent documents dated 4/30/03, must be used for consenting study subjects. This informed consent form has been stamped by the IRB office and will expire on April 15, 2004. You may not use this informed consent document after its expiration date. You must submit a progress report (Continuing Review Form) sufficiently (one to two months) prior to this date of expiration of your study to permit review by the IRB. You will receive a new informed consent document to use for the following year if the Continuing Review of your project is approved.

Final approval to initiate this study has been given previously. You are authorized to conduct the research project as detailed in your protocol. Any proposed changes in the research study must be submitted, reviewed and approved by the IRB before the change can be implemented. The only exception is a change necessary to eliminate apparent immediate hazards to the research subjects. In such a case, the IRB should be promptly informed of the change following its implementation for IRB review.

You must conduct this research, and supervise the research staff involved in this research project in accordance with Federal, State, Local and Institutional policies concerning the use of human subjects in research. In conducting this research you must comply with IRB policies detailed in the most recent version of the IRB Policies and Procedures. If you do not have a copy of the IRB Policies and Procedures, do not continue this research, contact the IRB office immediately to receive it and review it carefully before you continue your research.

ORIGINAL ARTICLE

Puberty and Genetic Susceptibility to Breast Cancer in a Case–Control Study in Twins

Ann S. Hamilton, Ph.D., and Thomas M. Mack, M.D., M.P.H.

ABSTRACT

BACKGROUND

Breast cancer is thought to result from excessive cumulative exposure to ovarian hormones. Different predictors of hereditary and sporadic breast cancer suggest different pathogenic mechanisms. Affected twin pairs may help to illustrate such differences.

METHODS

We obtained information from 1811 pairs of female twins, one or both of whom had breast cancer. The pairs were stratified according to concordance or discordance for breast cancer, zygosity, the presence or absence of a family history of breast cancer, and the presence of bilateral or unilateral disease. Disease-concordant monozygotic pairs were assumed to have a higher genetic susceptibility than other subgroups of pairs. Paired twins were compared with respect to age at puberty and other factors. We calculated adjusted odds ratios for the diagnosis of breast cancer when only one twin was affected and for the first of the two diagnoses when both were affected.

RESULTS

Within disease-discordant monozygotic pairs, the twin with an earlier onset of puberty did not have an increased risk of breast cancer (adjusted odds ratio, 0.8; 95 percent confidence interval, 0.6 to 1.2). Within disease-concordant monozygotic pairs, the twin with earlier puberty was much more likely to receive the diagnosis first (adjusted odds ratio, 5.4; 95 percent confidence interval, 2.0 to 14.5). In contrast, a later first pregnancy, lower parity, and later menopause within the pair were associated with an increased risk of breast cancer when one twin was affected but did not predict an earlier diagnosis when both were affected.

CONCLUSIONS

Within the most genetically susceptible subgroup of twin pairs, the strong influence of earlier puberty on the age at the diagnosis of breast cancer and the absence of linkage to hormonal milestones later in life suggest that most cases of hereditary breast cancer are not related to cumulative hormone exposure and that they may instead result from an unusual sensitivity to pubertal hormones. Associations between breast cancer and early menarche and those with reproductive milestones in adulthood may reflect different genotypes.

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N Engl J Med 2003;348:2313-22.

BREAST CANCER CAN RESULT FROM THE actions of ovarian hormones[1] that stimulate cell proliferation[2] and that may increase the rate of genetic errors in ductal cells. This view is based on hormonal risk factors, a "breast-age" (hormonal) index that predicts age-specific incidence,[3,4] and differences in plasma estrogen levels between patients with cancer or their family members and controls.[5,6] Even perinatal[7-9] and environmental[10-12] risk factors have been attributed to hormonal differences. Genetic risk factors include those that regulate the production, transport, and metabolism of estrogens[13]; determine the hormonal sensitivity of cells[14]; or repair errors of replication.[15]

Observation of affected pairs of twins[16] followed prospectively for new diagnoses showed a constant and much higher age-specific incidence of breast cancer throughout adulthood in the identical twins of women with cancer than in similar women in the general population.[17] Moreover, the overall level of risk was more than twice that conferred by disease in a first-degree relative, constituting a much greater increase than would be produced by single dominant alleles.[18] We postulated that genetically determined breast cancer accounts for a larger proportion of the total number of cases than previously thought and that most such cancers result from two or more individually low-penetrance allelic variants coexisting in a highly penetrant combination. We interpreted the constant, age-specific pattern of risk in identical twins of patients with breast cancer to be inconsistent with causation by cumulative exposure to hormones.

On the basis of the very high relative and cumulative risk to a woman who is genomically identical to a woman with cancer, disease in monozygotic twins who are both affected is considered largely to represent hereditary cancer, whereas disease in only one twin of a pair is believed to represent sporadic, or less heritable, disease. Cases among disease-discordant dizygotic pairs represent the same mixture of heritable and sporadic cases as those seen in ordinary case–control studies. The current analysis is based on a previously described population[17] and includes all twins in affected pairs who completed a risk-factor questionnaire. To determine whether risk factors differed according to genetic susceptibility, we stratified pairs on the basis of zygosity, concordance or discordance of disease, the presence of bilateral or unilateral disease, and the presence or absence of a family history of breast cancer.

In contrast to a conventional analysis of case–control pairs, an analysis of pairs concordant for disease may seem unusual, since few differences in exposure might be expected. We hypothesized that an earlier exposure to hormones or exposure to a higher level of hormones might be linked to an earlier diagnosis of breast cancer.

METHODS

From 1980 to 1991, 17,245 twin pairs responded to advertisements in North American periodicals seeking "twins with cancer and other chronic diseases."[16] Among the 6325 female twin pairs were 2718 women with breast cancer, among them women in 200 monozygotic and 109 dizygotic disease-concordant pairs (i.e., pairs in which both twins were affected). The affected pairs were followed at regular intervals until February 1993 to identify additional cancer diagnoses in the women with a previous diagnosis and new diagnoses in the unaffected twins. By then, an additional 77 monozygotic and 22 dizygotic disease-concordant pairs had been identified. Assessment of ascertainment bias, diagnostic validation, and assignment of zygosity has been reported elsewhere.[16,18]

At ascertainment, questionnaires concerning risk factors for breast cancer were sent to the 4241 living members of 2475 affected female twin pairs. Women from 1944 of these pairs (78.5 percent) replied. Responses from 1811 pairs (759 dizygotic and 1052 monozygotic) were considered sufficient for analysis. Each woman was asked about her twin as well as about herself, an approach that permitted assessment of pairs even when only one of the twins responded. Proxy responses were found to be biased only with respect to events occurring late in life.[19]

Standard case–control questions (e.g., about the age at menarche) were posed, as were questions unique to twin studies, calling for a comparative response with ranking of the relative magnitude or sequence of an exposure within the pair. Odds ratios were computed for each variable after the exclusion of pairs of twins who disagreed on the ranking of that variable and, in the context of menopausal variables, pairs in which only one of the twins responded.

Within each zygosity group, three strata were defined according to the probable level of genetic susceptibility: twins discordant for breast cancer, with no evidence of genetic or familial risk; twins discordant for cancer but with bilateral disease in

the affected twin or a history of breast cancer in another (nontwin) first-degree relative; and twins concordant for breast cancer. Pairs were analyzed as matched sets with the use of conditional logistic regression (PROC PHREG program, SAS Institute), with adjustment of odds ratios for potentially confounding variables. Heterogeneity between strata was assessed by determining (with use of the Wald test) whether the ratio of frequencies constituting the two odds ratios was statistically compatible with unity.

RESULTS

As we have previously reported,[18] more monozygotic pairs than dizygotic pairs were concordant for breast cancer (20 percent vs. 12 percent) (Table 1). Among disease-concordant pairs, monozygotic and dizygotic pairs had a similar prevalence of factors associated with an increased genetic risk of breast cancer. Among disease-discordant monozygotic and dizygotic pairs, 20.9 percent and 24.3 percent, respectively, reported at least one such factor. Although a young age at diagnosis and Jewish ethnicity have been linked to *BRCA1* and *BRCA2* mutations, neither of these factors was significantly more prevalent among pairs with evidence of genetic risk (data not shown).

Within concordant pairs, the mean interval between the twins' diagnoses varied little according to zygosity (8.6 years in dizygotic pairs and 7.4 years in monozygotic pairs). No significant overall differences between twins within the groups stratified according to zygosity or disease concordance were seen with respect to mean parity or with respect to age at menarche, at the onset of breast development or menstrual regularity, at the time of the first full-term pregnancy, or at menopause (Table 2).

Table 1. Concordance for Breast Cancer and Factors Related to the Genetic Risk of Breast Cancer among Twin Pairs, According to Zygosity.*

Variable	Dizygotic Twins	Monozygotic Twins
	no. of pairs (%)†	
Concordance for breast cancer		
Discordant	670 (88.3)	843 (80.1)
Concordant	89 (11.7)	209 (19.9)
Total	759	1052
Level of genetic risk		
Discordant for breast cancer		
Bilateral disease (GR+)	40 (6.0)	34 (4.0)
Breast cancer in another first-degree (nontwin) relative (GR+)	109 (16.3)	129 (15.3)
Both of the above (GR+)	14 (2.1)	13 (1.5)
Neither of the above (GR–)	507 (75.7)	667 (79.1)
Total	670	843
Concordant for breast cancer (all GR++)		
Bilateral disease in one or both twins	15 (16.8)	41 (19.6)
Breast cancer in another first-degree (nontwin) relative	15 (16.8)	40 (19.1)
Both of the above	9 (10.1)	16 (7.7)
Neither of the above	50 (56.2)	112 (53.6)
Total	89	209

* The genetic risk of breast cancer was assessed according to predefined terms at the outset of the study. GR– indicates no evidence of genetic risk (i.e., twins discordant for cancer, without bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), GR+ a possible increase in genetic risk (i.e., twins discordant for cancer but with bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), and GR++ a probable increase in genetic risk (i.e., twins concordant for cancer).

† Because of rounding, not all percentages total 100.

ONSET OF PUBERTY

Both direct measures and comparative measures (i.e., those referring to the within-pair rank order of events) with respect to age at menarche were obtained. In more than two thirds of the monozygotic and dizygotic pairs, the twins agreed that puberty had come later in one of the twins than in the other (more than one year later in 47.7 percent of the monozygotic pairs and 71.3 percent of the dizygotic pairs).

Most indicators of earlier puberty in one of the two twins were strongly and significantly associated with breast cancer in the discordant dizygotic pairs with some evidence of genetic risk (Table 3). Among those without evidence of genetic risk, the same associations were weaker and were confined to pairs in which the affected twin received the diagnosis before the age of 50 years. The same measures predicted the earlier diagnosis within concordant, dizygotic pairs; earlier menarche was a significant factor when the first diagnosis came before the age of 50 years (Table 3).

When discordant monozygotic pairs were assessed, no link with earlier puberty was apparent in the pairs with no evidence of genetic risk, and only

Table 2. Age at the Time of Occurrence of Selected Variables and Parity before the Diagnosis of Breast Cancer, According to Zygosity and Concordance for Breast Cancer.*

Variable	Dizygotic, Pairs Discordant for Cancer		Dizygotic, Pairs Concordant for Cancer	Monozygotic, Pairs Discordant for Cancer		Monozygotic, Pairs Concordant for Cancer
	Unaffected Women	Women with Cancer		Unaffected Women	Women with Cancer	
Breast development						
Age — yr	12.3 (12.2–12.4)	12.2 (12.0–12.3)	12.1 (11.9–12.3)	12.2 (12.1–12.3)	12.2 (12.1–12.3)	12.2 (12.0–12.4)
No. of women	557	541	137	741	714	337
Menarche						
Age — yr	12.8 (12.7–12.9)	12.7 (12.6–12.8)	12.7 (12.4–12.9)	12.8 (12.6–12.8)	12.8 (12.7–12.8)	12.8 (12.6–12.9)
No. of women	625	591	154	804	768	379
Menstrual regularity						
Age — yr	13.2 (13.0–13.3)	13.0 (12.9–13.2)	12.8 (12.4–13.1)	13.1 (12.9–13.2)	13.1 (13.0–13.2)	13.2 (13.0–13.4)
No. of women	546	490	128	678	636	321
First full-term pregnancy						
Age — yr	24.2 (23.8–24.5)	24.3 (24.0–24.8)	24.9 (24.0–25.8)	24.5 (24.2–24.9)	24.8 (24.5–25.2)	25.4 (24.9–26.0)
No. of women	500	485	129	630	623	301
Parity						
Value	2.3 (2.2–2.4)	2.2 (2.1–2.4)	2.1 (1.9–2.4)	2.3 (2.2–2.4)	2.2 (2.1–2.3)	2.0 (1.9–2.2)
No. of women	660	657	176	834	831	412
Menopause†						
Age — yr	43.6 (42.7–44.4)	43.5 (42.6–44.4)	42.8 (40.9–44.8)	44.6 (43.7–45.3)	44.7 (43.9–45.5)	44.4 (43.2–45.5)
No. of women	306	266	74	365	357	164

* The values for age and parity are means, with 95 percent confidence intervals given in parentheses.

† Only women in whom menopause occurred before their diagnosis of breast cancer (or the diagnosis in their twins, for unaffected women) were included in this analysis. Women who had had a hysterectomy without oophorectomy were excluded.

moderate associations were seen within pairs with some degree of genetic risk (Table 3). However, within concordant monozygotic pairs, every indicator of earlier puberty in one twin than in the other strongly, consistently, and significantly predicted the first diagnosis of breast cancer. These indicators included earlier development of breasts (about six months before menarche, as also reported elsewhere[20]) (Table 2), earlier menarche (whether according to a comparative or a direct response on the questionnaire), menarche before the age of 12 years, and earlier menstrual regularity (on average, six months after menarche). When we constructed a summary index of earlier puberty according to the concurrence of at least two of these indicators, the twin with earlier puberty was 5.4 times as likely to receive the first diagnosis of breast cancer (95 percent confidence interval, 2.0 to 14.5).

The interval between the twins' age at menarche did not influence the strength of the link between earlier puberty and an earlier breast-cancer diagnosis (Table 3), but when the first menarche in the pair occurred before the age of 12 years, the association between earlier puberty and an earlier diagnosis was more than three times as strong as it was when puberty occurred at the age of 12 years or later (adjusted odds ratio, 3.1; 95 percent confidence interval, 1.3 to 7.6) (Table 4). In that circumstance, the association between an early-puberty index that was greater than 1 and an earlier diagnosis reached 9.1 (95 percent confidence interval, 1.1 to 77.1).

DEVELOPMENTAL AND OTHER REPRODUCTIVE VARIABLES

Within concordant monozygotic pairs, greater height and weight during childhood (known predictors of early puberty) in one twin than in the other were associated with an earlier diagnosis of breast cancer (Table 5). A history of more medical problems at birth or during infancy in one twin than in the other was related to an increased risk of breast cancer within discordant pairs and to an earlier diagnosis within concordant dizygotic (but not monozygotic) pairs. Within discordant monozygotic pairs

Table 3. Puberty-Related Risk Factors and Adjusted Odds Ratios for Breast Cancer or First Breast Cancer among Twins, According to Zygosity, Concordance for Breast Cancer, and Level of Genetic Risk.*

Risk Factor	Dizygotic, Discordant for Cancer		Dizygotic, Concordant for Cancer	Monozygotic, Discordant for Cancer		Monozygotic, Concordant for Cancer
	GR–	GR+	GR++	GR–	GR+	GR++
Diagnosis at any age						
No. of pairs	508	163	88	667	176	209
First menarche (comparative response)	1.1 (0.9–1.3)	1.4 (0.9–2.0)	1.4 (0.9–2.4)	1.0 (0.8–1.2)	0.8 (0.5–1.1)	1.6 (1.2–2.3)†
First menarche (direct response)						
By 1 yr	1.1 (0.8–1.5)	1.6 (0.9–2.9)	1.6 (0.7–3.5)	1.1 (0.8–1.4)	1.2 (0.7–2.2)	1.4 (0.8–2.3)
By ≥2 yr	1.2 (0.8–1.6)	1.5 (0.9–2.7)	1.6 (0.6–4.3)	0.6 (0.4–1.1)	0.8 (0.3–2.2)	1.4 (0.6–3.3)
By any interval	1.1 (0.9–1.4)	1.6 (1.0–2.4)	1.6 (0.8–3.0)	1.0 (0.8–1.2)	1.1 (0.7–1.8)	1.4 (0.9–2.2)
Age at menarche <12 yr	1.1 (0.7–1.6)	2.2 (1.1–4.4)	2.3 (0.8–6.6)	0.8 (0.5–1.3)	0.6 (0.2–1.7)	3.0 (1.2–7.8)†
First breast development	1.0 (0.8–1.3)	1.7 (1.1–2.6)	1.8 (0.9–3.3)	0.8 (0.6–1.1)	0.8 (0.4–1.6)	3.6 (1.7–7.7)†
First menstrual regularity	1.1 (0.8–1.4)	1.3 (0.8–1.9)	1.2 (0.6–2.1)	1.0 (0.8–1.3)	1.0 (0.6–1.6)	2.4 (1.5–3.7)†
Early-puberty index >1‡	1.0 (0.7–1.3)	1.4 (0.8–2.3)	1.4 (0.7–3.0)	0.8 (0.6–1.2)	0.8 (0.3–1.9)	5.4 (2.0–14.5)†
Age at diagnosis						
No. of pairs with first diagnosis <50 yr	262	87	52	324	89	115
No. of pairs with first diagnosis ≥50 yr	244	75	34	341	87	93
First menarche (comparative response)						
<50 yr	1.3 (0.9–1.8)	1.3 (0.8–2.1)	1.3 (0.7–2.4)	1.0 (0.7–1.2)	0.9 (0.5–1.5)	1.4 (0.9–2.2)
≥50 yr	0.9 (0.6–1.2)	1.4 (0.8–2.6)	1.9 (0.8–4.5)	1.1 (0.8–1.4)	0.6 (0.3–1.1)	2.1 (1.2–3.6)†
First menarche (direct response)						
<50 yr	1.3 (0.9–1.8)	1.2 (0.7–2.1)	3.0 (1.2–7.0)	1.0 (0.7–1.4)	1.5 (0.8–2.9)	1.1 (0.6–2.0)
≥50 yr	1.0 (0.7–1.4)	2.2 (1.1–4.4)	0.6 (0.2–1.9)	0.9 (0.7–1.3)	0.8 (0.4–1.7)	1.8 (0.9–3.5)
Age at menarche <12 yr						
<50 yr	1.2 (0.7–2.0)	2.4 (0.9–5.9)	3.4 (0.9–12.7)	0.8 (0.4–1.6)	0.7 (0.2–2.3)	4.3 (1.2–15.6)†
≥50 yr	0.9 (0.5–1.8)	2.0 (0.7–5.8)	0.5 (0.1–6.5)	0.7 (0.3–1.6)	0.6 (0.1–4.0)	1.7 (0.4–7.3)
First breast development						
<50 yr	1.2 (0.9–1.7)	1.6 (0.9–2.8)	1.4 (0.7–3.1)	0.7 (0.5–1.1)	1.2 (0.5–3.0)	2.6 (1.1–6.4)†
≥50 yr	0.8 (0.6–1.2)	1.9 (0.8–4.2)	2.4 (0.7–7.9)	0.9 (0.6–1.5)	0.4 (0.1–1.2)	7.9 (1.7–36.2)†
First menstrual regularity						
<50 yr	1.3 (0.9–1.7)	1.1 (0.7–1.9)	1.0 (0.5–2.0)	0.9 (0.6–1.2)	1.3 (0.7–2.5)	1.8 (1.1–3.2)
≥50 yr	0.9 (0.6–1.3)	1.5 (0.8–2.9)	1.8 (0.5–6.2)	1.1 (0.8–1.6)	0.6 (0.3–1.4)	3.5 (1.7–7.4)†
Early-puberty index >1‡						
<50 yr	1.2 (0.8–1.8)	1.4 (0.7–2.6)	1.1 (0.4–2.6)	0.8 (0.5–1.4)	1.3 (0.4–4.4)	5.2 (1.4–18.6)†
≥50 yr	0.7 (0.4–1.1)	1.4 (0.6–3.3)	3.1 (0.6–15.9)	0.8 (0.4–1.6)	0.3 (0.1–1.4)	5.8 (1.2–27.4)†

* All variables were adjusted for nulliparity and age at first full-term pregnancy (age, ≤25 yr vs. >25 yr) and variables other than those related to first menarche, first breast development, and first menstrual regularity were also adjusted for first menarche. Numbers in parentheses are 95 percent confidence intervals. GR– indicates no evidence of genetic risk (i.e., twins discordant for cancer, without bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), GR+ a possible increase in genetic risk (i.e., twins discordant for cancer but with bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), and GR++ a probable increase in genetic risk (i.e., twins concordant for cancer).

† $P<0.05$ for the comparison between the concordant pairs (GR++) and discordant pairs (GR–).

‡ An early-puberty index of greater than 1 was defined as the presence of two or more of the following: first menarche, first breast development, or first menstrual regularity.

Table 4. Menarche-Related Risk Factors and Adjusted Odds Ratios for Breast Cancer or First Breast Cancer among Twins, According to Zygosity, Concordance for Breast Cancer, and Level of Genetic Risk.*

Risk Factor	Dizygotic, Discordant for Cancer		Dizygotic, Concordant for Cancer	Monozygotic, Discordant for Cancer		Monozygotic, Concordant for Cancer
	GR–	GR+	GR++	GR–	GR+	GR++
No. of pairs	421	135	68	574	164	171
First menarche in pair and age at first menarche						
<12 yr	1.1 (0.8–1.7)	1.9 (0.9–3.7)	2.0 (0.7–5.6)	1.0 (0.6–1.5)	0.6 (0.2–1.6)	3.1 (1.3–7.6)†
≥12 yr	1.1 (0.8–1.5)	1.3 (0.7–2.2)	1.3 (0.6–3.0)	1.0 (0.7–1.3)	1.6 (0.9–2.8)	1.0 (0.6–1.6)
≥13 yr	1.2 (0.8–1.8)	1.8 (0.8–3.9)	1.1 (0.3–3.8)	1.3 (0.8–2.1)	1.6 (0.7–3.5)	1.1 (0.5–2.6)
Early-puberty index >1 and age at first menarche‡						
<12 yr	1.0 (0.6–1.7)	1.6 (0.7–3.4)	2.2 (0.6–8.6)	0.7 (0.3–1.5)	1.0 (0.2–5.2)	9.1 (1.1–77.1)†
≥12 yr	1.0 (0.7–1.6)	1.3 (0.6–2.7)	1.0 (0.3–3.0)	1.0 (0.6–1.5)	0.7 (0.2–2.1)	4.6 (1.3–17.0)
≥13 yr	0.9 (0.5–1.7)	1.9 (0.7–5.4)	0.2 (0.1–1.4)	0.9 (0.4–1.8)	0.7 (0.2–2.5)	2.8 (0.5–14.8)

* All variables were adjusted for nulliparity and age at first full-term pregnancy (≤25 yr vs. >25 yr). Numbers in parentheses are 95 percent confidence intervals. GR– indicates no evidence of genetic risk (i.e., twins discordant for cancer, without bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), GR+ a possible increase in genetic risk (i.e., twins discordant for cancer but with bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), and GR++ a probable increase in genetic risk (i.e., twins concordant for cancer).

† P<0.05 for the comparison between concordant pairs (GR++) and discordant pairs (GR–).

‡ An early puberty index of greater than 1 was defined as the presence of two or more of the following: first menarche, first breast development, or first menstrual regularity.

of twins, but not within other pairs, earlier first full-term pregnancy and higher parity in one twin were significantly associated with a reduced risk of breast cancer (adjusted odds ratio for each of these factors, 0.7; 95 percent confidence interval, 0.5 to 0.9).

Within each subgroup of discordant pairs of dizygotic twins, earlier menopause (either by natural causes or by bilateral oophorectomy) and fewer reproductive years conferred the expected protection against breast cancer (Table 6). No such effects were seen within concordant monozygotic pairs. Use of estrogen-replacement therapy had no effect in any subgroup, but use of such therapy was of short duration in most of the participants.

DISCUSSION

Our findings support the hypothesis that the risk of breast cancer for a genetically susceptible woman is determined not by cumulative exposure to ovarian hormones but rather by exposure to the flood of hormones present at puberty. Among monozygotic twins, breast cancer that occurred within disease-concordant pairs was more likely than not to be heritable, and breast cancer that occurred within disease-discordant pairs was more likely than not to represent the sporadic form. Within genetically susceptible twin pairs, the first twin to experience puberty, especially if she did so before the age of 12, was much more likely to be the first twin to receive a diagnosis of breast cancer at a later date. If these disease-concordant monozygotic pairs had been interviewed just after the first diagnosis (permitting a comparison between the initial case and the as yet unaffected twin control), evidence of earlier puberty would have ranked among the strongest predictors of breast cancer. Within these pairs, the factors usually found to be the strongest predictors — age at first full-term pregnancy, parity, and age at menopause — were completely unrelated to the sequence of diagnoses.

In contrast, within the discordant monozygotic pairs, composed of twins with sporadic cases and controls, the pattern of risk was reversed. By any measure, relative age at puberty was unrelated to the risk of breast cancer. Moreover, sporadic breast cancer was linked to each reproductive factor during adulthood, suggesting that these cancers may be caused by higher cumulative exposure to ovarian hormones over a lifetime.

Table 5. Developmental and Reproductive Risk Factors and Adjusted Odds Ratios for Breast Cancer or First Breast Cancer among Twins, According to Zygosity, Concordance for Breast Cancer, and Level of Genetic Risk.*

Risk Factor	Dizygotic, Discordant for Cancer		Dizygotic, Concordant for Cancer	Monozygotic, Discordant for Cancer		Monozygotic, Concordant for Cancer
	GR–	GR+	GR++	GR–	GR+	GR++
No. of pairs	507	163	88	667	176	209
Developmental factors						
Greater weight at birth	1.0 (0.8–1.3)	0.9 (0.6–1.3)	1.1 (0.7–1.9)	0.8 (0.7–0.9)	1.1 (0.8–1.6)	1.1 (0.8–1.5)
First to walk	1.1 (0.8–1.5)	1.1 (0.6–1.8)	0.8 (0.4–1.9)	1.0 (0.7–1.5)	0.8 (0.4–1.8)	0.9 (0.5–1.9)
More medical problems than other twin						
At birth	1.0 (0.8–1.4)	1.3 (0.8–2.1)	2.5 (1.2–5.5)†	1.6 (1.2–2.2)	1.9 (1.1–3.3)	1.1 (0.7–1.9)
During infancy	1.3 (0.9–1.8)	1.4 (0.8–2.3)	1.7 (0.8–3.4)	1.2 (0.8–1.6)	2.0 (1.1–3.9)	1.1 (0.6–2.0)
Greater height						
At 10 yr	1.3 (1.1–1.6)	0.6 (0.4–0.9)	1.3 (0.8–2.2)	0.8 (0.6–0.9)	1.3 (0.8–2.0)	1.5 (0.9–2.3)†
At 20 yr	1.2 (0.9–1.5)	0.8 (0.5–1.1)	1.1 (0.7–1.8)	0.8 (0.6–1.0)	1.2 (0.8–1.9)	1.0 (0.7–1.6)
Greater weight						
At 10 yr	1.1 (0.9–1.4)	0.8 (0.5–1.1)	0.8 (0.5–1.5)	0.7 (0.6–0.9)	1.1 (0.7–1.8)	1.5 (0.9–2.4)†
At 20 yr	1.0 (0.8–1.2)	1.0 (0.7–1.5)	0.9 (0.6–1.6)	0.8 (0.6–0.9)	0.7 (0.5–1.1)	1.1 (0.7–1.6)
Greater body-mass index						
At 20 yr	1.3 (1.0–1.7)	1.4 (0.9–2.0)	1.0 (0.5–2.1)	0.9 (0.7–1.1)	0.7 (0.4–1.1)	0.9 (0.6–1.4)
At 40 yr	1.3 (1.0–1.7)	1.2 (0.7–1.9)	0.7 (0.4–1.4)	1.2 (0.9–1.5)	0.7 (0.5–1.1)	1.2 (0.8–1.9)
Reproductive factors						
Age at first full-term pregnancy						
≤25 yr	0.9 (0.7–1.3)	0.8 (0.4–1.4)	1.2 (0.5–2.8)	0.7 (0.5–0.9)	1.1 (0.6–2.0)	1.2 (0.7–2.1)
>25 yr	1.0	1.0	1.0	1.0	1.0	1.0
Nulliparity	1.2 (0.8–1.8)	1.6 (0.8–3.2)	1.4 (0.6–3.2)	1.0 (0.7–1.5)‡	0.7 (0.4–1.3)	1.9 (0.9–3.9)‡
Parity						
Nulliparity	1.2 (0.8–1.8)	1.6 (0.8–3.3)	1.3 (0.6–3.0)	1.0 (0.7–1.5)	0.6 (0.3–1.2)	2.0 (0.9–4.0)
1 or 2	1.0	1.0	1.0	1.0	1.0	1.0
≥3	1.0 (0.7–1.4)	0.8 (0.4–1.5)	1.0 (0.5–2.1)	0.7 (0.5–0.9)‡	1.0 (0.6–1.6)	1.3 (0.8–2.1)
Current or previous use of oral contraceptives	1.0 (0.7–1.5)	1.8 (0.8–3.8)	2.2 (0.6–7.6)	1.1 (0.8–1.5)	0.9 (0.5–1.6)	1.1 (0.6–2.1)

* All variables were adjusted for nulliparity and age at first full-term pregnancy (≤25 yr vs. >25 yr); variables other than those related to first menarche, first breast appearance, and first menstrual regularity were also adjusted for first menarche. Numbers in parentheses are 95 percent confidence intervals. GR– indicates no evidence of genetic risk (i.e., twins discordant for cancer, without bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), GR+ a possible increase in genetic risk (i.e., twins discordant for cancer but with bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), and GR++ a probable increase in genetic risk (i.e., twins concordant for cancer).

† $P<0.05$ for the comparison between the concordant pairs (GR++) and the discordant pairs (GR–).

‡ $P<0.05$ for trend.

Taken together, our findings suggest that the relatively minor increase in risk seen after early menarche in population-based studies reflects an average of risks derived from a minority with a strong, genetically determined susceptibility to the hormonal milieu of puberty and a majority without such susceptibility. If we had not stratified the twins in our study according to genetic risk, the overall relative risk associated with the first menarche within the pair would have been 1.2 (95 percent confidence interval, 1.0 to 1.3), a risk similar to that found in many population-based studies.

If an onset of puberty that is 7.2 months earlier, on average, in one twin than in the other within a

Table 6. Menopause-Related Risk Factors and Adjusted Odds Ratios for Breast Cancer or First Breast Cancer among Twins, According to Zygosity, Concordance for Breast Cancer, and Level of Genetic Risk.*

Risk Factor	Dizygotic, Discordant for Cancer†		Monozygotic, Discordant for Cancer		Monozygotic, Concordant for Cancer
	GR–	GR+	GR–	GR+	GR++
No. of pairs	170	66	301	82	90
Menopausal status at diagnosis					
Before menopause	1.0	1.0	1.0	1.0	1.0
After natural menopause	0.7 (0.3–1.9)	0.5 (0.1–2.4)	1.2 (0.5–2.6)	1.1 (0.2–6.6)	0.4 (0.1–2.0)
After bilateral oophorectomy	0.6 (0.2–1.5)	0.6 (0.1–2.7)	0.7 (0.4–1.5)	1.6 (0.3–8.8)	1.5 (0.4–4.5)
Age at menopause‡					
First group (youngest)	1.0	1.0	1.0	1.0	1.0
Second group	1.3 (0.4–4.1)	1.6 (0.3–9.1)	1.3 (0.6–2.8)	1.1 (0.1–8.9)	0.3 (0.1–1.9)
Third group (oldest)	4.8 (1.6–14)§	4.4 (0.8–26)	1.1 (0.5–2.8)	0.8 (0.1–5.4)	0.2 (0.1–1.4)
Later menopause than other twin	1.6 (0.9–2.5)	1.1 (0.5–2.3)	1.0 (0.7–1.4)	0.6 (0.2–1.2)	0.9 (0.4–1.7)
By 1 or 2 yr	0.6 (0.2–1.3)	0.3 (0.1–1.3)	0.7 (0.4–1.2)	0.5 (0.2–1.7)	1.8 (0.6–5.1)
By 3–6 yr	1.8 (0.8–3.9)	2.2 (0.6–7.7)	1.0 (0.5–1.8)	0.3 (0.1–1.4)	0.5 (0.1–2.2)
By ≥7 yr	4.2 (1.5–11)	1.9 (0.5–7.8)	2.0 (0.9–4.4)	1.3 (0.3–5.5)	0.5 (0.1–1.7)
Reproductive period before diagnosis¶					
First group (shortest)	1.0	1.0	1.0	1.0	1.0
Second group	2.5 (0.9–6.2)	1.0 (0.2–4.6)	1.1 (0.5–2.4)	0.6 (0.2–2.5)	1.1 (0.3–4.4)
Third group (longest)	3.9 (1.4–11)§	3.2 (0.7–13)	1.3 (0.5–3.1)	0.4 (0.1–2.0)	0.4 (0.1–1.8)
Longer reproductive period than other twin	1.8 (1.2–2.6)	1.6 (0.8–3.1)	0.9 (0.6–1.2)	0.8 (0.5–1.5)	0.9 (0.5–1.5)
Current or previous use of estrogen-replacement therapy	1.0 (0.4–2.2)	1.1 (0.4–3.1)	1.1 (0.6–1.9)	0.5 (0.1–2.4)	0.9 (0.4–2.4)

* All variables were adjusted for first menarche, nulliparity, and age at first full-term pregnancy (≤25 yr vs. >25 yr). Numbers in parentheses are 95 percent confidence intervals. GR– indicates no evidence of genetic risk (i.e., twins discordant for cancer, without bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), GR+ a possible increase in genetic risk (i.e., twins discordant for cancer but with bilateral disease in the affected twin or a history of breast cancer in another [nontwin] first-degree relative), and GR++ a probable increase in genetic risk (i.e., twins concordant for cancer). Data shown include only double-respondent pairs in which each twin was premenopausal, had undergone natural menopause, or had undergone bilateral oophorectomy.

† Data for disease-concordant dizygotic pairs are not shown because the sample was too small.

‡ The three groups for age at menopause were defined on the basis of the controls' age distribution, as follows: discordant dizygotic, GR–: <43 yr, 43–47 yr, and ≥48 yr; discordant dizygotic, GR+: <42 yr, 42–46 yr, and ≥47 yr; discordant monozygotic, GR–: <42 yr, 43–48 yr, and ≥49 yr; discordant monozygotic, GR+: <43 yr, 43–49 yr, and ≥50 yr; concordant monozygotic: <44 yr, 44–49 yr, and ≥50 yr.

§ $P<0.05$ for trend.

¶ The three groups for length of reproductive period were defined on the basis of the controls' distribution, as follows: discordant dizygotic, GR–: <30 yr, 30–34 yr, and ≥35 yr; discordant dizygotic, GR+: <29 yr, 29–33 yr, and ≥34 yr; discordant monozygotic, GR–: <29 yr, 29–35 yr, and ≥36 yr; discordant monozygotic, GR+: <31 yr, 31–35 yr, and ≥36 yr; and concordant monozygotic: <31 yr, 31–36 yr, and ≥37 yr.

genetically identical pair can lead after decades to a diagnosis of heritable breast cancer that is 7.4 years earlier, on average, very-long-term consequences depend on a genetic factor that acts no later than at puberty. This factor could affect only the hormonal milieu of puberty or the cellular response to it. Because puberty marks a brief period of great proliferation and differentiation in the epithelial and stromal cells of the breasts,[21] a heritable factor related to cellular susceptibility provides the plausible explanation. Increases in gonadal hormone production continue into adulthood,[22] and any heritable alteration would not be likely to act at such an early age. Hormone levels could not be directly measured in our study, but empirical evidence of an unusual hormonal milieu, early or late, was not apparent. Although women with an earlier menarche tend to have higher estrogen levels than those with a later menarche,[23] in our study, the group of twins with hereditary breast cancer was similar to the group

with sporadic breast cancer in terms of parity, age at menarche, age at the time of breast development, age at the time of initial menstrual regularity, and age at menopause. Finally, no reproductive variable occurring in adulthood in the twins we studied predicted the earlier diagnosis. As a result, we favor the hypothesis that much of the genetic susceptibility to breast cancer derives from a pathologic cellular response to the physiologic increase in the production of hormones at puberty.

Although few of the women with hereditary breast cancer in our study were tested for the dominant mutations in BRCA1 or BRCA2,[18] these allelic variants probably do not explain their genetic susceptibility. Most received the diagnosis after the age of 40 years, and their tumors tended to be estrogen-receptor–positive rather than estrogen-receptor–negative.[18] Moreover, the proportion of twins who were Jewish was not especially high (9.6 percent and 7.5 percent of concordant and discordant monozygotic pairs, respectively), and only 3.8 percent of concordant monozygotic pairs reported a twin or other first-degree relative with ovarian cancer — a proportion similar to that in other subgroups of twins.

We cannot identify a study artifact that might explain our results. The twins were ascertained as case–control pairs, matched according to the level of motivation to participate in the study and other unmeasurable confounding factors. When we included known, measurable confounding variables in the analysis (including the variables reported above, as well as height in childhood and adulthood, body-mass index and body weight at 20 and 40 years of age, change in body-mass index between these ages, and use or nonuse of alcohol and tobacco), the results did not change. Questionnaire responses within twin pairs were unrelated to their prior perceptions of the cause of their breast cancer. When the women were asked to speculate about the causes of breast cancer, "stress" was the most common response, and women from concordant monozygotic pairs were not more likely than others to mention "hormonal factors." Because of concern about possible nonindependence of responses, we requested the women first to complete the questionnaire independently in black ink and then to use red pens (which were provided) if they wished to change an answer. Twins in less than 1 percent of all the pairs made changes with regard to age at menarche.

The inclusion of pairs in which diagnoses of breast cancer were ascertained retrospectively could theoretically have resulted in the omission of women with a short survival, provided that the surviving twin preferentially chose not to participate in the study. Such omission appears to have been unlikely, however, since survivors were represented among the participants in expected proportions[16] and, in any case, since women who had died were included by means of proxy information. Moreover, we compared the results from concordant monozygotic pairs identified prospectively with the results from pairs identified retrospectively. Of the 34 concordant monozygotic pairs in which the twins differed according to the early puberty index, 14 were identified before the second case was diagnosed. The adjusted odds ratio for early puberty derived solely from this prospective subgroup (4.9; 95 percent confidence interval, 1.1 to 22.4) is similar to that based solely on the subgroup of pairs identified retrospectively (6.7; 95 percent confidence interval, 1.8 to 25.2).

In a study of twin pairs in Scandinavia and Great Britain that were discordant for breast cancer, more rapid prepubertal growth and earlier breast development were predictive of breast cancer, although small numbers precluded stratification according to genetic risk.[24] Age at menarche has been found to predict breast cancer in some studies of women with breast cancer who have a family history of the disease[25,26] but not in other such studies,[27,28] and age at menopause has been found to be unrelated to breast cancer in the presence of a family history.[29] Even so, such results cannot be directly compared with our findings, since family history alone was used in those studies to identify women with cancer who were at high genetic risk; women with cancer associated with genes of low penetrance and combinations of alleles inherited from different parents would have been excluded.

Thus, a major form of hereditary breast cancer may be triggered by unusual sensitivity to the rush of hormones at puberty. Such a genetic sensitivity to hormone exposure has been observed in rats: estradiol treatment promotes breast cancer only in certain strains.[30] Modification of the risk of breast cancer according to age at the time of hormone exposure is also not an unprecedented finding. The carcinogenic action of ionizing radiation on the breast is magnified by early age at exposure,[31] and the protection against induced breast cancer in rats given genistein, a soy isoflavonoid, is enhanced when it is given before puberty.[32]

If a substantial subgroup of women in the general population is at higher risk for breast cancer than the rest of the population because of very early puberty, then as the age at puberty continues to decline in the population, this subgroup may become increasingly prominent. Only when the pertinent genotype or genotypes become known can methods of intervention and detection of hereditary breast cancer be devised. Genomic material from identical twins who are concordant for disease should greatly facilitate that search.

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